RESIDUES AND TRACE ELEMENTS

Multivessel Supercritical Fluid Extraction of Food Items in Total Diet Study

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An off-line, large capacity, multivessel supercritical fluid extractor (SFE) was designed and constructed for extraction of large samples. The extractor can simultaneously process 1–6 samples (15–25 g) by using supercritical carbon dioxide (SC-CO_2), which is relatively nontoxic and nonflammable, as the solvent extraction medium. Lipid recoveries for the SFE system were comparable to those obtained by blending or Soxhlet extraction procedures. Extractions at 10 000 psi, 80°C, expanded gaseous CO_2 flow rates of 4–5 L/min (35°C), and 1–3 h extraction times gave reproducible lipid recoveries for pork sausage (relative standard deviation [RSD], 1.32%), corn chips (RSD, 0.46%), cheddar cheese (RSD, 1.14%), and peanut butter (RSD, 0.44%). In addition, this SFE system gave reproducible recoveries (>93%) for butter fortified with *cis*-chlordane and malathion at the 100 ppm and 0.1 ppm levels. Six portions each of cheddar cheese, saltine crackers, sandwich cookies, and ground hamburger also were simultaneously extracted with SC-CO_2 and analyzed for incurred pesticide residues. Results obtained with this SFE system were reproducible and comparable with results from organic-solvent extraction procedures currently used in the Total Diet Study; therefore, use and disposal of large quantities of organic solvents can be eliminated.

The Total Diet Study (TDS) (1) of the U.S. Food and Drug Administration (FDA) has been investigating off-line supercritical fluid extraction (SFE) as an alternative extraction technique for table-ready (peeled, cooked, etc.) food items with 2% or more fat. SFE offers a cost saving in the initial extraction steps of these methods because large quantities of organic solvents can be eliminated. A total of 119 fat food items (containing 2% or more of fat) of a possible 261 table-ready food items are contained in the TDS. These fat food items are analyzed 4 times a year for pesticide residues at the 0.001 ppm level. The 119 food items are analyzed by multiresidue methods described in the FDA's *Pesticide Analytical Manual* (PAM; 2).

Recent literature articles demonstrate that SFE is a viable alternative extraction technique for pesticides in general (3–8), pesticides from grain and crop matrixes (9), and lipid moieties (10–12). However, a need exists for a technique and associated apparatus to extract 15–25 g samples because these samples are representative of sample food matrixes for TDS.

The supercritical carbon dioxide (SC-CO_2) extraction technique described by Hopper and King (13) exhibits the capabilities needed for extracting pesticides and matrix components on the previously mentioned scale from the 119 fatty food items (>2% fat) in the TDS. These food items contain various amounts of moisture (0–87%). Pesticides and fat matrix components are extracted efficiently with SC-CO_2 after being mixed with an extraction enhancer (14), which disperses the sample and absorbs water.

The combined volume of the larger sample size and added extraction enhancer necessitated development of an SFE system with a large capacity extraction vessel. A large capacity (98 mL extractor volume), off-line, multivessel SFE system was designed and constructed because no existing commercial equipment met these specifications. The basic concept of the multivessel SFE system developed by King and co-workers (15, 16) at the U.S. Department of Agriculture was the basis of the current instrument.

Experimental

Principle

Fatty food samples were mixed with the extraction enhancer and poured into the extraction vessel. Samples were then extracted with SC-CO_2, and the fatty residue containing the pesticides was collected. A portion of the precipitated residue was

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cleaned up by gel permeation and Florisil adsorption chromatography. Organochlorine pesticide residues were determined by gas chromatography (GC) with an electron capture detection (ECD) system and an electrolytic conductivity detection (ELCD) system in the halogen mode. Organophosphate residues were determined by GC and flame photometric detection (FPD) in the phosphorus mode. The amount of fat in each food item was determined gravimetrically.

Apparatus and Reagents

(a) *Extraction apparatus.*—Extractions were performed with the apparatus shown in Figures 1 and 2. Commercial grade liquid carbon dioxide (LCO_2) from a cylinder overpressurized with helium at 1 300 psi (Linweld Gas Supply, Kansas City, MO) was fed through a cylinder connection fitting (CGA 320 × 1/4 in. NPT; Matheson Gas Products, Gloucester, MA) attached to a stainless steel flex hose (6042, Matheson Gas Products), 3-way valve (3K63; Butech, Erie, PA), coupler (SS-400-6; Swagelock Co., Solon, OH), and 4-CS Cajon cross (Swagelock).

The LCO_2 passed through the cross and was split into 2 paths. One path went through a tee (20T4; Butech) to a relief valve (20SH4-1/4A, 2000 psi rupture disk; Butech) and then to a solenoid valve (4A691; Dayton Mfg. and Electric Co., Chicago, IL), which was used to cool the pump head. The second path went through a CO_2 cleanup trap (*see* following description) and a check valve (20BC4; Butech) before entering the pump (Model DSHF-151C with modifications 17860-28881-29376; Haskel Engineering Corp., Burbank, CA). Compressed CO_2 exited the pump into a connecting line through a tee (20T4) fitted with a relief valve (20SH4-1/4A, 15 000 psi rupture disk) and then into a main shutoff valve (20UV44V) connected to a manifold consisting of tees (20T4), an elbow (20L4), a cross (20 × 4), a 20 000 psi system pressure gauge (20PG-PM) fitted with an adapter (10A4H12P), and 6 flow control/shutoff valves (20MV46V) (Butech). The system extraction pressure was set to the desired value (10 000 psi) by adjusting the air regulator that controlled the air pressure to the pump. House air was supplied to the pump through a filter (A963DX; Balston Air Filter, Lexington, MA).

Compressed CO_2 exited the 6 flow control/shutoff valves at ambient temperature through a bulkhead coupling (20BF4), a check valve (20BC4), a tee (20T4), an adapter (MA4M4M) (Butech), and then to each extraction vessel it served. The extraction vessels were mounted vertically in a large extraction oven (LCA2-12; Despatch Industries, Inc., Lakeville, MN). Extractor vessels consisted of 316 SS tubing (20-562-316; reducing couplings, 20F4M16M; Butech) pressure-rated to 20 000 psi at 54.2°C and having dimensions of 2.54 cm od × 1.43 cm id × 60.96 cm (98 mL). Vessels were machined on one end to accept a 10 µm SS frit (1000-.625-.125-10-A; Mott Metallurgical Corp., Farmington Industrial Park, Farmington, CT). The extraction fluid was equilibrated to the 80°C oven temperature by passing it through a 1 m length of interconnecting

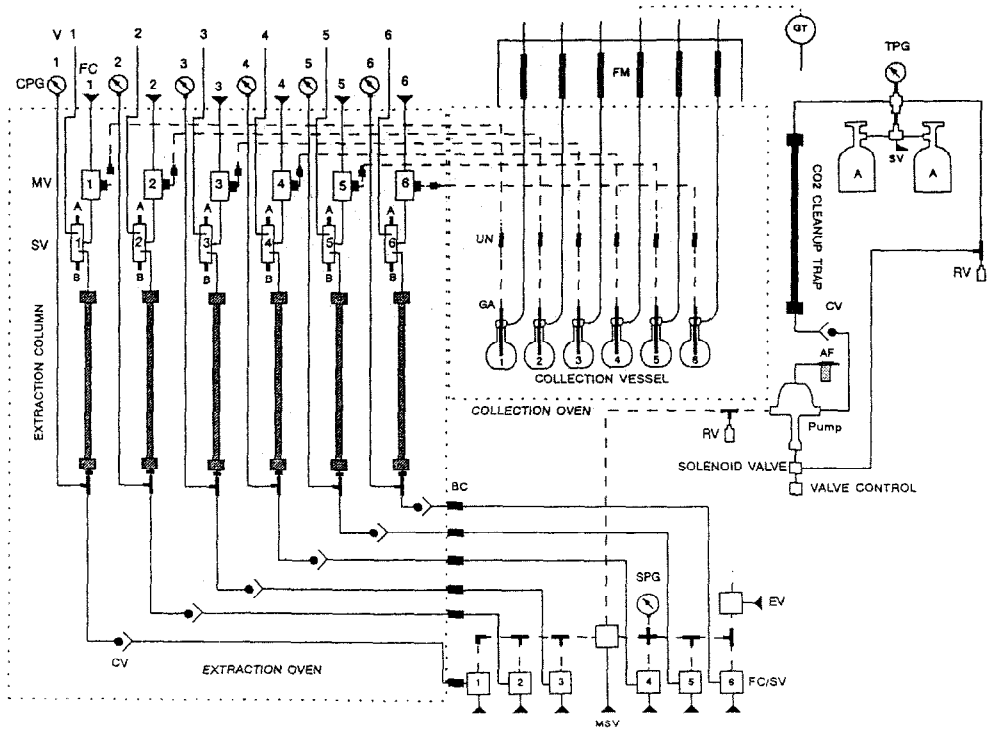

Figure 1. SFE system: dashed lines indicating a box are thermostated regions; A, cylinder; SV, switch valve; TPG, tank pressure gauge; CV, check valve; AF, air filter; RV, relief valve; MSV, main shutoff valve; SPG, system pressure gauge; FC/SV, flow control/shutoff valve; SV/A, column vent; SV/B, column shutoff; EV, exhaust valve; BC, bulkhead coupling; MV, micrometering valve; UN, union; GA, glass adaptor; CPG, column pressure gauge; FC, flow control; V, vent; FM, flow meter; and GT, gas totalizer.

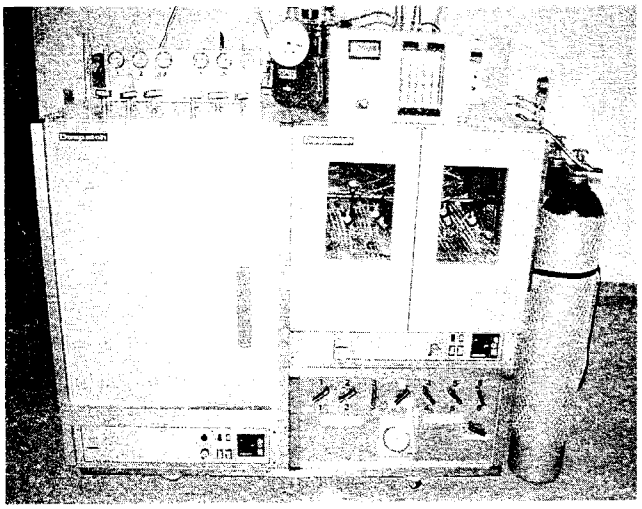

Figure 2. Supercritical fluid extractor.

tubing between the check valve and the bulkhead coupling. The solute-laden SC-CO_2 passed from the extraction vessel (frit end) through a 2-stem valve (20UV45V; Butech) into a micrometering valve (Autoclave 30VRMM, fitted with adapters 20M 44B6 and 15M 42BI), where decompression of SC-CO_2 occurred.

Sudden decompression of SC-CO_2 created rapid cooling of the micrometering valve. Hence, the valves were heated to the extraction oven temperature (80°C), which prevented plugging of the orifice and the attached exit line. Interconnecting lines between the major components of the extractor, except from the micrometering valve, consisted of 316 SS, 0.32 cm od × 0.277 cm id tubing rated to 20 000 psi at 54.2°C (BT 20-109-316; Butech). Column pressure gauges (WIKA No. 233.30; Boston Cooper Corp., Salem, NH), each fitted with an adapter (10F4M4P), were used to monitor the individual extraction vessel pressure taken at the head of the column through a tee (BT 20T4) (Butech).

Gaseous CO_2 and extract passed through a reducing coupler (LPA4M2L) into a 2.54 cm × 0.3175 cm od × 0.1575 cm id piece of SS tubing (LP125-062-316) (Butech). The CO_2 and extract then passed through a reducing union (SS-200-6-1; Swagelok Co.), a 91.4 cm × 0.0762 cm id × 0.158 cm piece of SS tubing (30161; Alltech, Deerfield, IL), a union (SS-100-6; Swagelock Co.), and a glass adapter (*see* following description). The CO_2 and extract then passed into a 250 mL Florence flask (6896-08; Ace Glass, Vineland, NJ) equipped with a Therm-o-Vac adapter (No. 14510-15; Berghof/America, Concord, CA) modified to accommodate the glass adapter. The extract was deposited in the receiver, and the gas was passed through Teflon tubing to a flow meter (Brooks Sho II 150 low-flow indicator 6 tube manifold 1370-GT valve, tube F-6-151 S.S.T. float, 3.4–34.8 standard L/min CO_2; Brooks Instrument Division, Hatfield, PA) and a gas totalizer (DTM 200A; American Meter Division, Philadelphia, PA) before being vented to the atmosphere. The union, flat-bottom collection flask, and connecting lines were contained in an oven (LDB1-69AD-3; Despatch Industries, Inc.) set at 35°C.

(**b**) *CO_2 cleanup trap.*—A trap for CO_2 purification was constructed from 316 SS tubing (20-562-316; reducing coupling, 20F4M16M; Butech) pressure-rated to 20 000 psi at 54.2°C; tubing dimensions were 2.54 cm od × 1.43 cm id × 60.96 cm machined on one end to accept a 10 µm SS frit (1000-.625-.125-10-A; Mott Metallurgical Corp.). The trap was filled with a mixture consisting of 24 g coconut charcoal and 48 g Alumina C (12103-99; Scientific Adsorbents, Inc., Atlanta, GA), which was held in place with the 10 µm SS frit and a plug of glass wool. CO_2 exited through the frit end of the trap. The charcoal and Alumina C were conditioned at 160°C for 18 h before being packed into the column. The cleanup trap was purged with liquid CO_2 before use.

(**c**) *Glass adapter.*—A glass adapter for the receiving flask was constructed as shown in Figure 3. A 130 mm × 6.5 mm od × 4 mm id piece of glass tubing was fused to a 80 mm × 7 mm od × 2 mm id piece of glass tubing. An 18 in. × 1/16 in. od × 0.030 in. id piece of SS tubing (30161; Alltech) was inserted through the tubing until it protruded 3 mm from the end of a 7 mm od × 2 mm id piece of glass tubing. The end of the glass tube was sealed around the SS tubing, and a piece of 16 mm × 7 mm id × 9 mm od glass tubing was attached to the glass sealed around the steel tube. A lip (11 mm diameter) was flared on the piece of glass tubing. A 4 mm diameter glass bead was placed at the end of the tubing against the end of the SS tube. The glass was heated and 2 dimples, 180° apart, were placed 6 mm from the end of the SS tube.

(**d**) *Florisil trap.*—A 316 SS column, 30.4 × 0.95 cm id, containing 5 g activated Florisil (ref. 2, sec. **121.3**) held in place by glass wool plugs was used as a trap in testing the purity of CO_2.

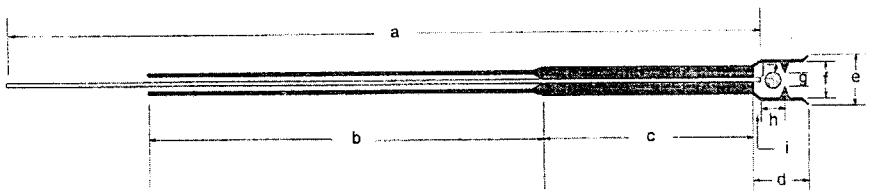

Figure 3. Glass adapter: a, 45.72 cm (18 in.) × 1.58 mm (1/16 in.) od × 0.762 mm (0.030 in.) id piece of stainless steel tubing; b, 130 mm × 6.5 mm od × 4 mm id piece of glass tubing; c, 80 mm × 7 mm od × 2 mm id piece of glass tubing; d, 16 mm; e, 11 mm; f, 7 mm; g, 3 mm; h, 6 mm; i, 3 mm; and j, 4 mm diameter glass bead.

(e) *Kuderna-Danish (K-D) concentrator.*—500 mL (K-570025-0500; Kontes, Vineland, NJ).

(f) *Snyder column.*—Three-ball (K-570000; Kontes).

(g) *Microevaporative concentrator.*—6709 (Ace Glass, Inc.).

(h) *Gel permeation chromatograph.*—The extracted fat from each sample was cleaned up on an Auto-Prep 1002B gel permeation chromatographic (GPC) system. The GPC system was equipped with a 60 cm × 2.5 cm id column (ABC Instruments [Laboratory Automation, Inc.], Columbia, MO), and slurry packed with 33 g Bio-Beads SX-3 resin (200–400 mesh; Bio-Rad Laboratories, Richmond, CA), and compressed to a bed length of ca 20 cm. The eluting solvent was methylene chloride–hexane (50 + 50, v/v) pumped at a flow rate of 5.0 mL/min and an operating pressure range of 8–11 psi. The GPC system was set up with a 12 min dump, 16 min collect, and 0 min wash cycle.

(i) *Gas chromatograph.*—The organochlorine pesticide residues were quantitated on a Model 5880 Hewlett-Packard GC system. The GC system was equipped with a 30 m × 0.32 mm DB-1701, 0.25 μm film thickness, fused-silica column (J&W Scientific, Folsom, CA) attached to a nickel-63 ECD system. The GC system was also equipped with a 30 m × 0.32 mm DB-5, 0.25 μm film thickness, fused-silica column (J&W Scientific) attached to a Model 4420 electrolytic conductivity detector (O.I. Analytical, College Station, TX). The GC system used a direct flash vaporization inlet with a retention gap connected to a quick-seal glass splitter. The hydrogen effluent from the splitter was attached to the fused-silica columns, and each column was connected to the specified detector.

(j) *Gas chromatograph.*—The organophosphate pesticide residues were quantitated on a Varian 3600 GC system. This system contained a 30 m × 0.53 mm DB-17, 1.0 μm film thickness, fused-silica column (J&W Scientific) attached to an FPD system operating in the phosphorus mode. The injection system incorporated a direct flash vaporization inlet. Each GC run consisted of a linear temperature program beginning at 150°C for 1 min, followed by a ramp to 230°C at 4°C/min, and finally a hold at 230°C for 20 min.

(k) *Extraction enhancer.*—Chem Tube Hydromatrix (0019-8004; Varian, Harbor City, CA) was passed through a 30 mesh sieve before being mixed with the samples. The fines passing through the sieve were discarded.

(l) *Florisil.*—PR grade, 60–100 mesh (Floridin Co., Berkeley Springs, WV); prepared as described previously (2).

(m) *Pesticide standards.*—Prepared from a 1 mg/mL stock solution dissolved in acetone–isooctane (10 + 90, v/v). All standards were obtained from the U.S. Environmental Protection Agency, Pesticide and Industrial Chemicals Repository, Research Triangle Park, NC.

(n) *Solvents.*—USP grade 95% ethanol and pesticide grade methylene chloride, *n*-hexane, diethyl ether, petroleum ether, acetonitrile, acetone, isopropyl alcohol, and isooctane were used in the procedures.

(o) *Alumina C.*—1 kg (12103-99; Scientific Adsorbents) preheated at 160°C for 18 h.

(p) *Coconut charcoal, activated.*—4 lb, 50–200 mesh (5-690-B; Fisher Scientific Co., Pittsburgh, PA) preheated at 160°C for 18 h.

(q) *Collection vessel.*—250 mL Florence boiling flask (6896; Ace Glass, Inc.).

(r) *Graduated mixing cylinders.*—25, 50, and 100 mL (8279-09, -12, and -15, respectively; Ace Glass, Inc.).

Sample Preparation

The fatty food items used in this study were obtained from TDS. Each item was cooked or prepared according to recipes used by the average American household. Large quantities of each prepared item were ground or blended into a composite. Each composite represented items purchased from 3 different locations within a specific region of the United States. The United States is divided by FDA into 7 regions, which are systematically sampled.

Sample Extraction

Samples ranging from 15 to 25 g and with 2–53% moisture were weighed into a 150 mL beaker and mixed by using a glass rod with 7–12 g sieved extraction enhancer until the mixture was homogeneous. Sample volume, sample moisture, and extraction vessel volume determined the amount of Hydromatrix needed to fill 95% of the vessel. The mixture was added through a powder funnel into the extraction vessel, which already contained 1 g extraction enhancer. The extraction vessel was loaded with the frit at the bottom (designated "top" down). The extraction enhancer at the top of the vessel retarded any water that may have solubilized during extraction. The vessel was tapped on the side with a rubber hammer to settle the mixture. An additional 1 g Hydromatrix was mixed in the original beaker and then transferred to the sample vessel and settled by tapping. This additional Hydromatrix assisted in removing any sample left in the beaker. The glass stirring rod and beaker were wiped with a plug of glass wool and added to the bottom of the vessel through the funnel. The high-pressure fittings were then assembled on the vessel, and the vessel was placed in the extraction apparatus with the end designated "top" upright in the extraction oven. The inlet line was attached to the vessel, and the vessel exit line was attached to the 2-stem valve. The extraction apparatus was pressurized to 10 000 psi and held in a static extraction mode for 15 min while the oven temperature reached 80°C. The CO_2 flow rate was set at 4–5 L/min (expanded CO_2 basis) for each column and adjusted, if necessary during extraction, to keep the flow rate constant. Each sample used volumes of CO_2 ranging from 227 to 755 L and was precipitated in a collection flask at atmospheric pressure and 35°C without any other solvents present. Extraction times varied from 1.25 to 3 h, depending on the item. Each extraction path from the vent line to the collection flask with extraction vessel isolated was flushed with 10 mL methylene chloride–hexane (50 + 50, v/v) at 5.0 mL/min.

Results and Discussion

The efficiency of the SFE pumping system was improved by increasing the head pressure of the LCO_2 tank from 900 to 1300 psi through helium addition and by cooling the pump head with a jet of LCO_2. The cooling system regulation for the pump was not precise, and the temperature fluctuated between 0° and 10°C. However, this temperature fluctuation did not affect the performance of the pump. The Haskel pump was able to deliver a total of 30 L/min of expanded gaseous CO_2 to all 6 extraction vessels, under the stated conditions, via intermittent cycling approximately every 3 s.

The large capacity off-line SFE system uses large quantities of CO_2; therefore, commercial LCO_2 was tested for use in the extractor after being put through the Alumina C and charcoal CO_2 cleanup trap. The CO_2 cleanup trap was placed between the LCO_2 tank and the pump inlet. Commercial grade CO_2 was checked for contaminants before and after the trap was packed. This check was facilitated by passing LCO_2 through the trap into a heated micrometering valve, where LCO_2 was decompressed and passed through the Florisil trap (as described previously) at atmospheric pressure. A total of 500 L of expanded gaseous CO_2 was passed through the Florisil trap.

After passage of CO_2, the Florisil trap was eluted with 50 mL petroleum ether followed by 50 mL acetone. Each eluate was concentrated to 1 mL and analyzed by GC–ELCD. Also, reagent blanks were prepared by eluting the solvents through the Florisil trap.

The chromatograms in Figure 4 show that halogenated contaminants can be removed from commercial grade LCO_2 by using the CO_2 cleanup trap with the mixture of Alumina C and coconut charcoal sorbents. Also, chromatograms from the acetone fraction show that other halogenated contaminants were removed by the trap (data not shown).

The chromatograms in Figure 5 compare the mini-Florisil eluate 1 from SFE analysis of cooked ground beef with the first eluate from TDS analysis by GC–ELCD. The SFE chromatogram (C1) represents 60 mg sample injected on-column, compared with the 25 mg sample equivalent injected on-column in TDS analysis (C2), which used conventional extraction procedures. In most cases, the difference in the SFE baseline from the original TDS analysis can be attributed to the increased sample equivalent injected. Also, chromatograms from eluates 2 and 3 of each analysis were similar. This similarity shows that cleaned up commercial grade LCO_2 can be used as an extraction fluid for this application. This capability is highly desirable because commercial grade LCO_2 is considerably less expensive than SFE grade CO_2 (17).

A glass adapter, shown in Figure 3, was constructed to reduce the aerosol produced upon decompression of CO_2. The flow of CO_2 suspended the glass ball against the end of the SS tubing because of the Bernoulli effect. Hence, the fat collected on the glass ball, and splattering of the collected fat was reduced. A small piece of glass wool was also placed in the sidearm of the Therm-o-Vac adapter to help collect any fat still suspended in the CO_2. This extractor was evaluated for analyte cross-contamination between vessels during simultaneous parallel extractions. Three 6 g portions of butter fortified with 100 ppm *cis*-chlordane and 100 ppm malathion were each mixed with 10 g Hydromatrix and extracted in vessels 2, 4, and 6. At the same time, three 6 g portions of unfortified butter were each mixed with 10 g Hydromatrix and extracted in vessels 1, 3, and 5. The results in Table 1 show that excellent recoveries can be obtained for samples fortified at 100 ppm with no attendant cross-contamination. This result was verified by GC–FPD and ELCD screening of the unfortified butter in vessels 1, 3,

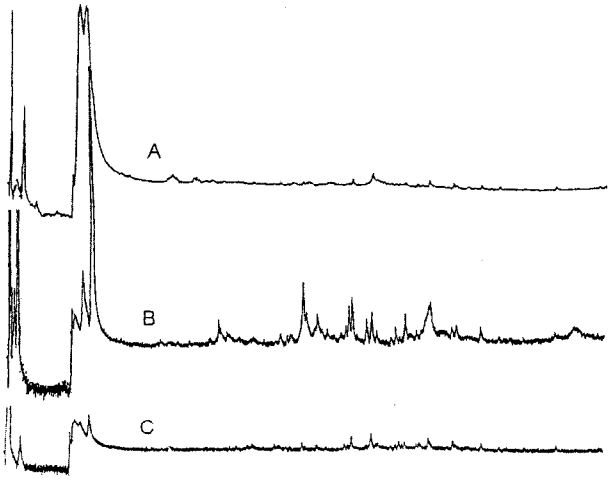

Figure 4. Chromatograms of petroleum ether eluates from different Florisil traps after CO_2 passage. Chromatogram A is from a CO_2 cleanup trap with charcoal and Alumina C, B is from a CO_2 cleanup trap without charcoal and Alumina C, and C is from a Florisil trap reagent blank.

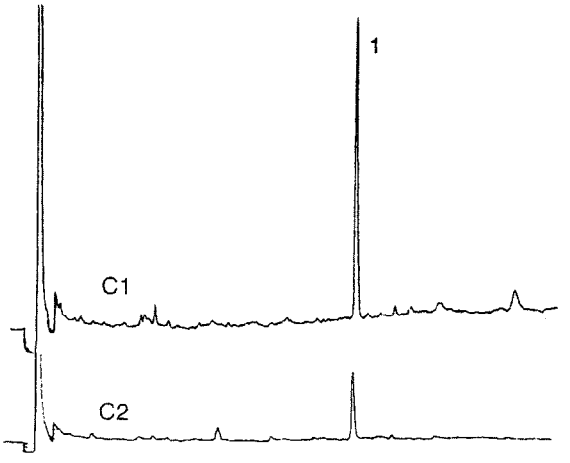

Figure 5. GC–ELCD chromatograms of mini-Florisil eluate 1 (20% methylene chloride in hexane) for extract from SFE (12.5 g/mL) and TDS (5 g/mL). Chromatogram C1 is from the SFE analysis using treated commercial grade supercritical CO_2 as extraction medium, and C2 is from the TDS analysis using organic solvents as extraction medium. Peak 1 at 12.37 min is *p,p*-DDE, an incurred pesticide residue at 0.009 ppm.

Table 1. Recoveries (%) for butter fortified with *cis*-chlordane and malathion at 100 ppm

	Vessel No.					
Pesticide	1	2	3	4	5	6
cis-Chlordane	0	95	0	96	0	93
Malathion	0	95	0	98	0	94

Table 2. Recoveries (%) for butter fortified with *cis*-chlordane and malathion at 0.10 ppm

Pesticide	Recovery range, vessels 1–6, %	Mean, % ($N = 6$)	RSD, %[a]
cis-Chlordane	95–101	99	2.2
Malathion	99–107	103	2.6

[a] RSD, relative standard deviation.

Table 4. Quantity of gaseous expanded CO_2 needed for fat yields comparable with those of PAM Vol. I, 211.13F, K, and C extraction procedures for the same matrixes

		Fat extracted	
Item	CO_2, L	Mean, %	RSD, %
Pork sausage ($N = 6$)	291	29.4	1.4
	460	29.8	1.3
Cheddar cheese, sharp/mild ($N = 6$)	200	32.5	1.5
	341	32.9	1.3
	451	33.3	1.1
Peanut butter ($N = 6$)	227	49.5	0.5
	361	49.5	0.4
Corn chips ($N = 5$)	173	28.7	1.0
	326	30.6	0.2
Corn chips ($N = 6$)	555	31.2	0.5
	755	31.5	0.5

and 5 for pesticides. No *cis*-chlordane or malathion was found above the 0.005 ppm limit of quantitation.

Sample-to-sample cross-contamination was eliminated by rinsing the system with a minimum of 8 mL methylene chloride–hexane (50 + 50, v/v) after each extraction. The flow path for each extraction vessel was rinsed with solvent through the following components: vent line, 2-stem manifold valve (extraction vessel isolated), micrometering valve, tubing, and connections into each collection flask. The collection path was then pressurized with nitrogen, and excess solvent was flushed into the receiver. In addition, the joint and side arm of the Therm-o-Vac adapter, and the glass adapter were each rinsed with 5.0 mL methylene chloride–hexane (50 + 50, v/v) to ensure complete sample recovery and no cross-contamination of analyte.

Each extraction vessel that previously had contained the 100 ppm fortifications was filled with 6 g unfortified butter mixed with 10 g Hydromatrix. The mixture was extracted without additional cleanup of the SFE unit. The extracted unfortified butter was analyzed for pesticides, and no *cis*-chlordane or malathion was found. Therefore, each extraction vessel can be used for future extractions without fear of contamination.

Six 5 g portions of a butter sample fortified with 0.1 ppm *cis*-chlordane and 0.1 ppm malathion were mixed with 10 g Hydromatrix. These samples were extracted simultaneously and analyzed. The results in Table 2 show that reproducible extractions (relative standard deviations [RSDs], of 2.2 and 2.6% and mean recoveries of 99 and 103%, respectively, for *cis*-chlordane and malathion) were achieved with the described extractor.

Lipid extractions were also performed on TDS composites of corn chips, cheddar cheese, peanut butter, and pork sausage to allow a comparison of different extraction procedures. Each of these composites, without further preparation, was extracted 6 times by using SC-CO_2 and traditional organic solvent-based methods. The amount of fat extracted with SC-CO_2 was compared with the amount extracted by Soxhlet (18) using petroleum ether or blending (PAM, Vol. 1, Section **211.13F**, **K**, and **C**) with petroleum ether or ethyl ethers.

The results from these experiments (Table 3) show that the SFE technique was reproducible; RSDs ranged from 0.4 to 1.3, and fat recoveries ranged from 98.2 to 100.4% compared with PAM, Vol. 1, Section **211.13F**, **K**, and **C** and from 97.4 to 100.6% compared with Soxhlet. Results in Table 4 show that varying amounts of SC-CO_2, 227–755 L gaseous CO_2, were needed to extract the same quantity of fat obtained via the PAM

Table 3. Comparison of fat extraction procedures[a] by percent fat extracted

	Method A		Method B		Method C		
Item	Mean, % ($N = 6$)	RSD, %	Mean, % ($N = 6$)	RSD, %	Mean, % ($N = 6$)	RSD, %	Hydromatrix wt., g
Pork sausage	30.6	4.8	29.8	1.6	29.8	1.3	7
Peanut butter, creamy	50.3	0.4	49.3	0.6	49.5	0.4	12
Cheese cheddar, sharp/mild	33.9	3.1	33.9	3.5	33.3	1.1	10
Corn chips	31.3	0.6	31.8	0.8	31.5	0.5	10

[a] Method A is AOAC method **960.39**, 4 g sample; Method B is PAM, Vol. 1, sec. **211.13F**, **C**, and **F**, 25 g sample; Method C is SFE method, 15 g sample.

Table 5. SFE analysis of samples with incurred pesticide residues

Parameter	Result of analysis by PAM I, **211.13**, ppm	SFE analysis		
		Range of incurred pesticide residue, ppm	Mean, ppm ($N = 6$)	RSD, % [a]
		Cheddar cheese[b]		
Heptachlor epoxide	0.001	0.0012–0.0013	0.0012	4.3
Dieldrin	0.001	0.0022–0.0029	0.0025	10.2
Fat, %	33.9		33.3	1.1
		Saltine crackers[c]		
Methyl chlorpyrifos	0.0230	0.0275–0.0381	0.0291	5.7
Malathion	0.0190	0.0300–0.0342	0.0316	4.5
Fat, %	10.0		8.9	0.8
		Sandwich cookies[d]		
Malathion	0.0310	0.0350–0.0390	0.0372	3.6
Fat, %	18.2		17.5	0.8
		Ground beef[e]		
p,p'-DDE	0.0070	0.0087–0.0093	0.0090	2.8
Fat, %	19.8		20.7	1.2

[a] RSD, relative standard deviation.
[b] Cheddar cheese, sharp/mild, 15 g sample (39% moisture), 10 g Hydromatrix/sample, 451 L CO_2/sample, PAM I method **211.13C**.
[c] Saltine crackers, 20 g sample (4% moisture), 7 g Hydromatrix/sample, 300 L CO_2/sample, PAM I method **211.13C**.
[d] Sandwich cookies, 25 g sample (2% moisture), 7 g Hydromatrix/sample, 307 L CO_2/sample, PAM I method **211.13C**.
[e] Ground beef, cooked, 25 g sample (53% moisture), 7 g Hydromatrix/sample, 300 L CO_2/sample, PAM I method **211.13F**.

Vol. I, **211.13F**, **K**, and **C** extraction procedures for these specific matrixes. Further extraction of samples with SC-CO_2 does not yield significant fat recovery.

Six portions of saltine crackers, sandwich cookies, and cooked ground hamburger were also extracted simultaneously by the SFE procedure; a total of 300 L expanded gaseous CO_2 was used for each extraction. The extracted fat from saltine crackers, sandwich cookies, ground hamburger, and cheddar cheese were analyzed for incurred pesticide residues. GPC (19), mini-Florisil (20), and an alternative elution system (PAM, Vol. 1, Section **252**) were used to clean up a portion of extracted fats before analysis. Eluates were analyzed for pesticide residues by GC using specific detectors previously described. Data in Table 5 show that this SFE procedure gave reproducible results, with RSDs ranging from 2.8 to 10.2%, which compared well with results from extraction procedures (PAM, Vol. 1, Section **211.13F**, **K**, and **C**) used in TDS.

Summary

This study shows that off-line, multivessel extraction using the described prototype extractor and SC-CO_2 is reproducible and comparable with organic solvent-based extraction. A unique advantage of this extractor is the ability to extract large sample amounts, which range from 15 to 25 g, such as the representative portions of food samples encountered in TDS analysis with mandated Soxhlet and blending techniques. The results show that this SFE system is viable for replacing the organic solvent extractions used in TDS to extract table-ready food items with >2% fat. Hence, the SFE system, when validated, will reduce organic solvent consumption and eliminate water–ether mixtures generated by the PAM Vol. I extraction procedures, which contribute significantly to TDS's hazardous waste. Future studies will focus on implementing this technique as a standard operating procedure for all 119 TDS fatty food items. Commercial parallel and sequential SFE extractors with less extraction vessel capacity are available.

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